European Review for Medical and Pharmacological Sciences 2023; 27: 5200-5210

The efficacy of metformin as adjuvant to chemotherapy on IGF levels in non-diabetic female patients with progressive and non-progressive metastatic breast cancer

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Abstract. – OBJECTIVE: Some studies have shown that metformin inhibits the proliferation of breast cancer (BC) cells *via* multiple ways. One of these mechanisms is through the indirect control of the IGF-route mediated *via* the activation of the AMPK-LKB1 pathway in the liver, which leads to a decrease in blood glucose and insulin levels. The objective of this study was to investigate the effects of metformin as adjuvant to chemotherapy on IGF levels in female patients with progressive and non-progressive metastatic BC.

PATIENTS AND METHODS: In this trial, 107 women receiving chemotherapy for metastatic breast cancer (MBC) were divided into two groups: the metformin group received 500 mg of metformin twice daily, whereas the control group did not receive any metformin. All patients received chemotherapy according to the South Egypt Cancer Institute's (SECI) established regimen. The level of IGF-1 was determined in the blood at the initiation of therapy (baseline) and at six months post treatment.

RESULTS: No substantial differences were noted regarding IGF-1 levels in both groups at baseline (IGF-1 average level was 40.74 ± 36.16 *vs.* 32.06 ± 20.00 in the metformin and the placebo group, respectively, $p = 0.462$). While after six months, the mean IGF-1 level was 37.62 ± 31.35 *vs.* 39.12 ± 2 5.93 in the metformin and placebo groups, respectively, ($p = 0.170$).

CONCLUSIONS: Metformin as an adjuvant to chemotherapy in MBC patients had no significant effect on reducing IGF-I levels which promotes the inhibition of the proliferation of BC cells in MBC patients.

Key Words:
Metformin, Metastatic breast cancer (MBC), IGF-1, Chemotherapy, Adjuvant therapy.

Introduction

Metastatic breast cancer (MBC) is a heterogeneous disease that is represented by a solitary metastatic lesion and metastasizes to various organs. Even though MBC remains incurable, there has been a notable rise in the overall survival (OS) rate among MBC patients[1].

Endocrine therapy, radiation, chemotherapy, bisphosphonates, and targeted therapy are all used to treat MBC. The choice of treatment is largely dependent on the status of the hormone

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receptor, disease site, and response to previous treatments. The goals of these therapies are to enhance patients' quality of life by reducing their symptoms and extending their OS and progression-free survival (PFS)[2].

Many studies[3,4] have revealed that a higher level of blood insulin-like growth factor-1 (IGF-1) and lower levels of its binding protein-3 (IGFBP-3) are linked with an increased risk of developing multiple tumors, including BC. IGF-1 is a multifunctional mitogenic peptide that promotes cancer cell growth, migration, transformation, differentiation, and proliferation[5,6]. It exerts its effect through direct activation of specific trans-membrane tyrosine kinase receptors that are overexpressed in BC, which raises the level of the insulin receptor-substrate-2 (IRS2) and activates the PI3K-Akt and MAPKinase pathways[7].

Hyperinsulinemia stimulates IGF-1 hepatic synthesis by decreasing hepatic production of IGF binding proteins (IGFBPs), leading to an increase in free bioactive IGF-1; thus, changes in circulating insulin concentration can influence the IGF-1 bioactivity[6,8]. Several studies[9,10] have demonstrated a link between type 2 diabetes mellitus and the development of BC. This association has been attributed to hyperinsulinemia which promotes the proliferation of cancer cells by activating insulin receptors (IRs) and/or increasing the bioactivity of IGF-1[11].

Nowadays, the oral hypoglycemic drug metformin has demonstrated a wide range of activity in cancer treatment due to its anti-tumorigenic effect against various types of cancers[12]. It was demonstrated, using tumor-induced models based on *in vivo* studies, that metformin inhibited BC cell proliferation *via* multiple mechanisms, including the indirect interference with the IGF-pathway mediated *via* activating the AMPK-LKB1 pathway in the liver, which leads to a reduction of circulating glucose level and hyperinsulinemia. Also, metformin was shown to directly exerts its potency *via* the activation of the AMPK pathway in neoplastic tissues. Both pathways stimulate the AMP-activated protein kinase to inhibit the mammalian target of rapamycin (mTOR), which mainly affects BC cell growth, apoptosis induction, and cell-cycle arrest[13].

Metformin as an anticancer medication in non-diabetic people is the subject of a few clinical investigations[13-16]. This study was conducted to investigate the efficacy of metformin as an adjuvant to chemotherapy on the IGF-1 levels in female patients with progressive and non-progressive metastatic BC.

Patients and Methods

Study Design

A prospective randomized clinical study was conducted on nondiabetic women with MBC receiving chemotherapy in accordance with the established standard protocol by the South Egypt Cancer Institute (SECI) in the period between June 2020 and July 2021. This study recruited 107 female patients with MBC and were divided into two groups. Group A (n = 57) received chemotherapy and metformin (500 mg twice daily), while group B (n = 50) received chemotherapy alone. This study aimed to assess the impact of metformin on IGF-1 levels.

Moreover, IGF-1 concentrations were assessed at baseline and after 6 months of treatment in both groups. ROC curve was used to show the ability of IGF-1 hormone for the prediction of progression disease among MBC patients. According to the cut-off value, IGF concentrations were divided into high IGF group and low IGF group. Furthermore, progression-free survival (PFS), which is defined as the duration from random assignment in a clinical study to disease progression or death from any cause, was assessed after 1 year of follow-up in both groups.

The Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria for target lesions were used to assess the tumor response rate. Regressive disease (RD) was defined as a reduction in the total of target lesions' diameters of at least 30% from the baseline. The sum of the diameters of the target lesions must be at least 20% larger than the smallest sum on study to be considered progressive disease (PD). Additionally, PD was considered when one or more new lesions appeared. Finally, stationary disease (SD) was recorded when, in comparison to the study's smallest sum diameters, there was neither adequate shrinkage to consider for RD nor sufficient growth to consider for PD[17].

Women who fulfilled the following criteria were eligible for inclusion: female patients with MBC, patients more than 18 years old, nondiabetic patients, patients received only chemotherapy, patients with non-metastatic BC, those with multiple cancers (double cancer), those undergoing hormone therapy or radiotherapy, those with diabetes, those with a history of cardiac illness, and those who were hypersensitive or allergic to metformin were not allowed to participate.

Laboratory Measurements

In both groups, around 2 mL of venous blood samples were collected in ethylenediamine

tetraacetic acid (EDTA) tubes at time zero (baseline) and six months following treatment. The blood samples were allowed to coagulate under room temperature (25°C), and the plasma was separated by centrifugation at 4,000 rpm for about 10-15 minutes, and then separated plasma was collected in Eppendorf tubes (EP). The collected samples were stored at -80°C freezer till the time for IGF-1 analysis.

Insulin-Like Growth Factor 1 (IGF-1) Analysis

Test principle

An ELISA kit was used (Elabscience, Cat. No. E-EL-H0086, Texas, USA), and the instructions set by the manufacturer were followed[18-23].

Kit Components and Storage

Samples were left for two hours to clot at room temperature or overnight at 4°C prior to centrifugation at 1,000×g for 15 min. The supernatant was collected, and the assay was immediately performed. Blood collection tubes were non-endotoxin, non-pyrogenic, and disposable.

Note for Sample

Samples were either analyzed within 7 days when stored at 4°C, or were divided up and stored at -20°C (≤ 1 month) or -80°C (≤ 3 months). Repeated freeze-thaw cycles were avoided.

Reagent Preparation

Prior to use, all reagents were brought to room temperature (18-25°C). The Microplate reader manual (Winooski, VT, USA) was used for setup and preheated for 15 min before measuring optical density (OD). With respect to washing buffer, concentrated wash buffer (30 mL) was diluted with sterile distilled or deionized water (720 ml) to prepare the wash buffer (750 mL). In the event of crystal formation in the concentrate, it was warmed in a 40°C water bath and gently blended until entirely dissolving the crystals.

The standard working solution was centrifuged at 10,000 × g for 1 min. Reference Standard & Sample Diluent (1.0 mL; Elabscience, Cat. No. E-EL-H0086, Texas, USA) were mixed and left to stand for 10 min and inverted gently numerous times. After complete dissolution, it was thoroughly mixed with a pipette. This reconstitution yielded a 100 ng/ml working solution. As required, serial dilutions were performed. The dilution gradient that was advised was as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/mL.

Dilution Method

Seven EP tubes were obtained, and then Reference Standard & Sample Diluent (500 µL) were added to each tube. About 500 µL of the 100 ng/mL working solution were pipetted to the first tube and mixed to yield a 50 ng/mL working solution. Following these steps, 500 µL of the solution were pipetted from the former tube into the new one. The last tube was considered as a blank.

Regarding the Biotinylated Detection Ab working solution, prior to the experiment, the needed quantity (100 µL/well) was determined. It was best to prepare somewhat extra than calculated. Prior to use, the stock tube was centrifuged, and the 100 × Concentrated Biotinylated Detection Ab (Elabscience, Cat. No. E-EL-H0086, Texas, USA) was diluted to 1 × working solution using Biotinylated Detection Ab Diluent.

Regarding the Concentrated HRP Conjugate working solution (Elabscience, Cat. No. E-EL-H0086, Texas, USA), the required amount was calculated before the experiment (100 µL/well). In preparation, slightly more than calculated was prepared. The 100 × Concentrated HRP Conjugate was diluted to 1 × working solution with Concentrated HRP Conjugate Diluent.

Assay Procedure

The Standard working solution was added to the first two columns. Each solution concentration was added in duplicate to one well each, side by side (100 µL for each well). The samples were placed in the other wells (100 µL for each well). The plate was sealed using the sealer included in the kit and incubated at 37°C for 90 minutes.

The liquid was removed from each well, but it was not washed. Biotinylated Detection Ab working solution (100 µL) was added to each well before being covered with the plate sealer and gently blended, and then incubated at 37°C for one hour.

This solution was aspirated from each well and washing buffer (350 µL) was added to each well and left for 1-2 minutes, before being aspirated from each well and patted dry with clean absorbent paper. This washing procedure was performed three times.

HRP Conjugate working solution (100 µL) was added to each well before being sealed with the plate sealer and then incubated at 37°C for 30 min. This solution was aspirated from each well, and the wash process was repeated five times as conducted in Biotinylated Detection Ab working solution.

Substrate reagent (90 µL) was added to each well before being covered with a new plate sealer and incubated at 37°C for 15 minutes, which was protected against light. The reaction time was extended or shortened based on the actual color change, up to 30 minutes. Stop Solution (50 µL) was added to each well following the exact order as the substrate solution. Finally, each well's optical density was read using a biotech epoch microplate reader (Winooski, VT, USA) (OD450 nm).

Calculation of Results

The average of the duplicated readings for each standard and sample was calculated, then the average zero standard optical density was subtracted. A four-parameter logistic curve was plotted on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis as shown in Figure 1.

If the samples had been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpassed the upper limit of the standard curve, we re-tested it with an appropriate dilution. The concentration in use was obtained by multiplying the theoretical concentration by the appropriate dilution factor.

Ethical Statement and clinical registration

The Faculty of Pharmacy's Research and Ethics Committee (REC), Beni-Suef University approved to conduct this study with ethical number (REC-H-PhBSU-21028) and was registered at clinical trial registry (clinical trial.gov; NCT05840068). The confidentiality of participants was wholly guaranteed during the study. The study was conducted following the good clinical practices stated by the Declaration of Helsinki. Participation was voluntary and written informed consent was acquired from all participants and their legal guardians.

Statistical Analysis

Data analysis was performed using the version 22 of the SPSS software (IBM Corp., Armonk, NY, USA). Data with non-normal distribution were expressed as mean ± standard deviation (± SD), or median and range, relative frequencies (percentages), as well as frequencies (number of cases), when appropriate.

Quantitative variables comparisons were made using the Student's *t*-test for data normal distribution, Mann-Whitney U test for data with non-normal distribution, as well as Kruskal-Wallis test for comparison of more than two continuous groups. Wilcoxon sign rank test was utilized to compare paired data (pre- and post-treatment) with non-normal distribution.

To compare categorical data, the Chi-square (χ^2) test was utilized. The exact test was used instead when the expected frequency was less than 5. Receiver Operating Characteristic Curve (ROC) analysis was used to find out the best cut-off values for validating the prediction of disease progression in MBC patients. Kaplan-Meier test was utilized to compare survival between the two studied groups. Significance was considered at *p*-value lower than 0.05.

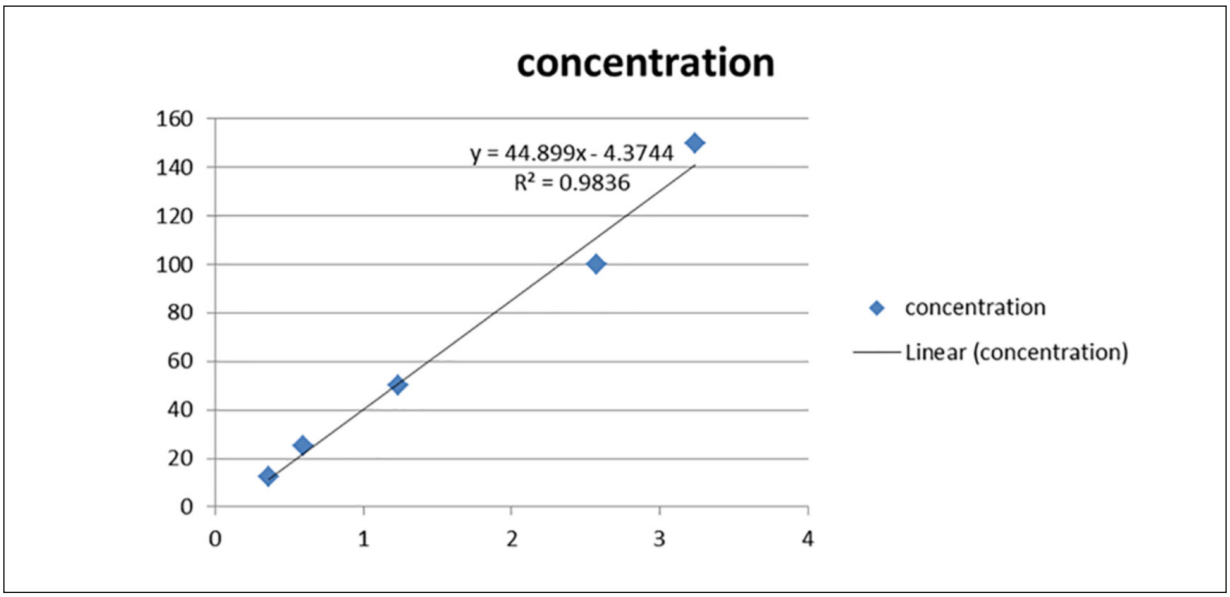

Figure 1. Standard curve of IGF concentrations.

Results

IGF-1 Analysis

Table I and Figure 2 illustrated the comparison of IGF-1 concentration at baseline and after 6 months of treatment between group A and group B. There was no significant difference between the two groups in IGF concentration at baseline (p^1 = 0.462). Also, there was no significant difference between the two groups in IGF concentration after 6 months (p^1 = 0.170) (p^1 indicated for the comparison between group A and group B in IGF level at baseline and after 6 months of treatment). There was no significant difference between IGF concentrations among patients in group A at baseline and after 6 months (p^2 = 0.357), while there was a significant difference between IGF concentrations among patients in group B at baseline and after six months (p^2 = 0.006) (p^2 indicated for comparing IGF level within the same group from before to after treatment). It was shown that IGF concentration of group B increased after six months as compared with its IGF concentration at baseline.

The Ability of IGF-1 for the Prediction of Disease Progression in MBC Patients Using ROC Curve

Table II and Figure 3 show the ability of IGF in predicting progressive disease among MBC patients using the ROC curve. The area under the ROC curve was 80.1%, which mean that IGF was observed to be a significant predictor of progression among metastatic BC patients (p = 0.000).

The Correlation Between IGF Biomarkers and Clinic-Pathological Data in Studied Subjects

The IGF concentrations were divided according to the above-mentioned cut-off value (17.1) into two categories (low IGF < 17.1, n = 25) and (high IGF ≥ 17.1, n = 73) as shown in Table III. Only the response rate was reported to be significant; the other variables, as stated in Table III, were not found to be significant when comparing the two groups. In low IGF MBC patients with RD response was about (24%) *vs.* (20.5%) in MBC patients with high IGF, while in MBC patients with SD response who had low IGF was

Table I. IGF-1 concentration among metastatic case at baseline (n = 107) and after six months of treatment (n = 98).

Variable name	Group A (CTH + metformin)	Group B (CTH alone)	p-value[1]
IGF-1 (at baseline), Mean ± SD	40.74 ± 36.16	32.06 ± 20.00	0.462
Median (range)	21.6 (9.1 - 114.9)	26.3 (6.4 - 87.4)	
IGF-1 (after 6 months), Mean ± SD	37.62 ± 31.35	39.12 ± 25.93	0.170
Median (range)	22.3 (3.0 - 107.1)	29.2 (7.9 - 133.0)	
***p*-value[2]**	0.357	**0.006***	

Quantitative data are presented in the form of mean ± SD and median (range). *Significance was set at $p < 0.05$. p-value[1]: comparing both groups. p-value[2]: comparing the same group from before to after treatment.

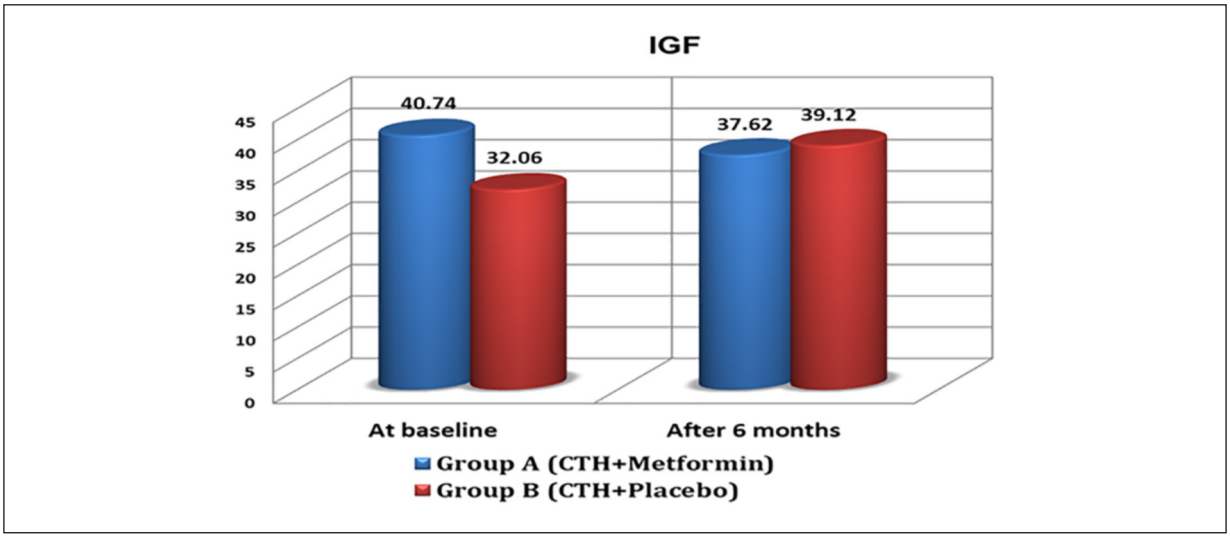

Figure 2. Bar graph showing the mean IGF between both studied groups at baseline and after 6 months of treatment.

Table II. The best cut-off, specificity, and sensitivity for predicting disease progression by IGF (n = 107).

	Cut off	95% CI	Sensitivity	Specificity	AUC	*p*-value
IGF-1	17.1	0.696 - 0.906	89.2%	66.7%	0.801	**0.000***

AUC: Area under the curve; CI: confidence interval. *Significance was set at $p < 0.05$.

about (64%) *vs.* (37%) in MBC patients with high IGF, while in MBC patients with PD response who had low IGF was about (12%) *vs.* (42.5%) in MBC patients with high IGF, with a significant difference between two groups ($p = 0.017$). According to BMI in both groups, the results showed that patients with BMI (< 25) were 9 (36%) in low IGF group *vs.* 23 (31.5%) in high IGF group, while patients with BMI (≥ 25 - < 30) were 8 (32%) in low IGF group *vs.* 22 (30.1%) in high IGF group, whereas patients with BMI (≥ 30) were 8 (32%) in low IGF group *vs.* 28 (38.4%) in high IGF group, with no significant difference between both groups ($p = 0.843$).

PFS According to IGF Biomarker

The mean of PFS at 1 year in low IGF group was about 60.0 ± 9.8% *vs.* 12.3 ± 3.8% in high IGF group, there was a significant difference between two groups as regard MBC patients with low IGF having better PFS than MBC with high IGF ($p = 0.000$) as shown in Figure 4.

Comparison of IGF Levels Based on Disease Progression

According to status of disease progression in MBC patients (group A and B), the two groups were divided into progressive and non-progressive patients as shown in Table IV. There was no significant difference between the mean of IGF concentration at baseline between progressive and non-progressive patients ($p^1 = 0.982$), while after 6 months, there was a significant difference between them ($p^1 = 0.000$).

There was a significant decrease in IGF concentration among non- progressive patients at baseline and after 6 months ($p^2 = 0.002$), while there was a significant increase in IGF concentration among progressive patients at baseline and after 6 months ($p^2 = 0.000$).

We note that all patients with progressive disease either in group A or B showed a significant increase in IGF level when compared to their baseline or to non-progressive patients.

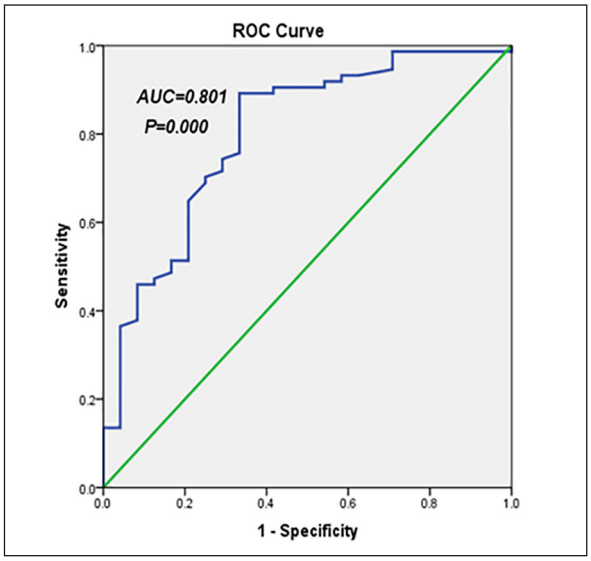

Figure 3. ROC curves for prediction of disease progression in patients with BC. IGF (blue) and reference line (green). Area under the curve = 0.801 (0.696-0.906), $p = 0.000$.

Discussion

According to the findings of this study, metformin had no therapeutic effect on IGF levels in chemotherapy-treated MBC patients. This observation may be attributed to the increase of progressing patients in both groups. There was a relationship between progressive disease and higher IGF-1 levels as IGF-1 activates tyrosine kinase receptor, which leads to the downstream activation of the PI3K-Akt and MAPKinase pathways, hence boosting cancer cell survival, proliferation, and resistance in BC[24]. In addition, several studies[25,26] on BC and colorectal cancer estimated that IGF-1/IGF-1 receptor (IGF-1R) signaling increased tumor associated lymph angiogenesis with lymphatic metastasis *via* the activation of vascular endothelial growth factor (VEGF). Furthermore, IGF-1 has been shown to perform a vital role in the activation of epithelial cancer cells to lose their polarity and gain the invasive and migratory properties of mesenchymal cells in a process known as epithe-

Table III. The correlation between IGF-1 biomarker and clinic-pathological details of the studied participants (n = 98).

Variable name		Low IGF (n = 25)		High IGF (n = 73)		*p*-value
Age (years)	**52.67 ± 14.53**	49.56 ± 12.69		0.431		
Grade	**Grade 2**	24	(96.0)	68	(93.2)	1
	Grade 3	1	(4.0)	5	(6.8)	
Menopausal status	**Premenopausal**	14	(56.0)	35	(47.9)	0.487
	Postmenopausal	11	(44.0)	38	(52.1)	
BMI	**< 25**	9	(36.0)	23	(31.5)	0.843
	≥ 25 - < 30	8	(32.0)	22	(30.1)	
	≥ 30	8	(32.0)	28	(38.4)	
ER	**Negative**	6	(24.0)	25	(34.2)	0.342
	Positive	19	(76.0)	48	(65.8)	
PR	**Negative**	10	(40.0)	30	(41.1)	0.923
	Positive	15	(60.0)	43	(58.9)	
Her2neu	**Negative**	14	(56.0)	42	(57.5)	0.894
	Positive	11	(44.0)	31	(42.5)	
Luminal A	**No**	14	(56.0)	38	(52.1)	0.733
	Yes	11	(44.0)	35	(47.9)	
Luminal B	**No**	17	(68.0)	56	(76.7)	0.388
	Yes	8	(32.0)	17	(23.3)	
Her2neu overexpression	**No**	22	(88.0)	59	(80.8)	0.548
	Yes	3	(12.0)	14	(19.2)	
Triple negative	**No**	22	(88.0)	66	(90.4)	0.712
	Yes	3	(12.0)	7	(9.6)	
Site of metastasis	**Visceral**	12	(48.0)	40	(54.8)	0.557
	Non visceral	13	(52.0)	33	(45.2)	
No. of metastatic sites	**1**	12	(48.0)	25	(34.2)	0.059
	2	12	(48.0)	29	(39.7)	
	> 2	1	(4.0)	19	(26.0)	
Response status	**RD**	6	(24.0)	15	(20.5)	**0.017***
	SD	16	(64.0)	27	(37.0)	
	PD	3	(12.0)	31	(42.5)	

Quantitative data are expressed mean ± SD; qualitative data are expressed as a number (percentage). *Significance set at $p < 0.05$.

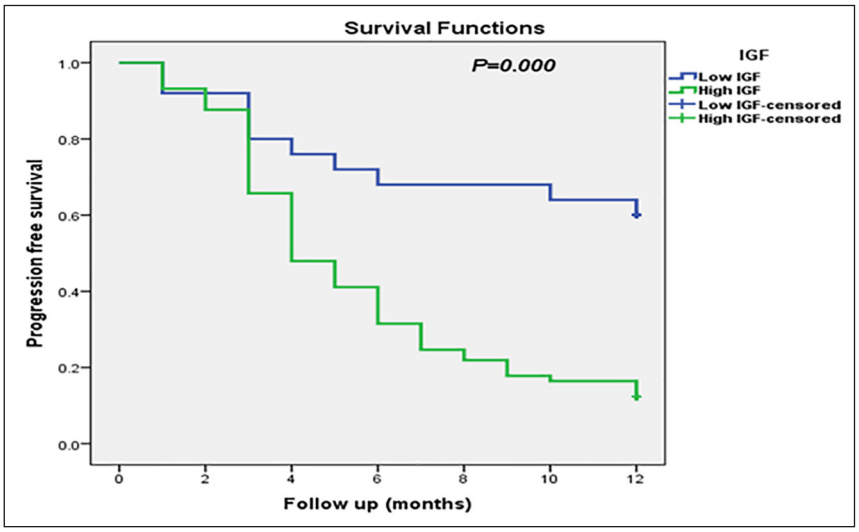

Figure 4. Kaplan-Meier curves for progression free survival among both studied groups according to IGF level.

Table IV. Comparison of IGF concentration according to disease progression status at baseline and after follow-up period.

Variable name	No. progression	Progression	*p*-value[1]
IGF-1 (at baseline), Mean ± SD	34.52 ± 27.09	37.31 ± 30.80	0.982
Median (range)	29.1 (9.1 - 111.1)	24.4 (6.4 - 114.9)	
IGF-1 (after 6 months), Mean ± SD	20.72 ± 18.22	43.91 ± 29.41	**0.000***
Median (range)	14.4 (6.5 - 90.3)	33.8 (3.0 - 133.0)	
***p*-value[2]**	**0.002***	**0.000***	

Quantitative data are expressed as mean ± SD and median (range). *Significance set at $p < 0.05$. *p*-value[1]: comparing both groups. *p*-value[2]: comparing the same group from before to after treatment.

lial to mesenchymal transition (EMT), which is regarded as one of the important programs in the metastatic cascade[27].

The present data is consistent with those of EL-Haggar et al[6], who illustrated that there was no significant difference in IGF levels between the metformin-treated group and the placebo group undergoing chemotherapy for patients who developed metastasis. According to the mean of IGF concentration, the present study showed that IGF concentration of the control group who received chemotherapy and placebo increased after 6 months as compared with its IGF concentration at the baseline, this may be attributed to the higher incidence of progressive disease in this group after 6 months.

The predictive ability of IGF in predicting progressive disease among MBC patients was assessed using the ROC curve analysis; showing a cutoff value of 17.1, the areas under the ROC curves of 80.1% (95%CI: 0.696 – 0.906) with a sensitivity of 89.2%, and specificity of 66.7%, which means that IGF was observed to be a significant predictor of progression among metastatic BC patients ($p < 0.001$). Such a finding may contribute to the major role of the insulin-like growth factor inhibitor (IGF-IR) in the proliferation of several types of cancer like pancreatic, colon, prostate, and breast cancer[27].

IGF-IR is composed of an intracellular β subunit which is responsible for signal transduction and an extracellular α ligand-binding subunit and binds to IGF-1 and IGF-2 ligand-activated IGF-IR. Therefore, the higher levels of IGF-I increase the risk of breast cancer as IGF-I overexpression leads to enhanced proliferation signals for the breast tumor, and develop resistance to cancer treatment, while inactivation of IGF-IR results in decreased growth and metastasis of breast tumor *in vivo*[29]. Further studies are required to confirm our observations. Bahhnassy et al[28], demonstrated that a cut off value of 106.96 ng/ml for serum IGF-I (93% sensitivity and 86.3% specificity) could be used to distinguish patients with triple negative breast cancer (TNBC) from those with non-triple negative breast cancer (non-TNBC). The difference between the two groups was statistically significant.

According to the cut-off value (17.1), the study subjects were divided into two main groups based on their IGF levels (low IGF < 17.1, n=25, and high IGF ≥ 17.1, n=73), and no significant difference was observed between both studied groups and clinic-pathological data. Regarding the triple negative patients, it was revealed that there was no significant difference between them ($p = 0.712$). There were about 3 patients (12.0%) in the low IGF group and 7 (9.6%) in the high IGF group. This finding conflicts with that of the Bahhnassy et al[28], which demonstrated that TNBC patients' IGF-I levels were significantly higher than those of non-TNBC patients. This may be attributed to smaller sample of triple negative patients collected in the present study.

According to BMI in both groups, the results showed that patients with BMI (< 25) were 9 (36%) in low IGF group *vs.* 23 (31.5%) in high IGF group, while patients with BMI (≥ 25 - < 30) were 8 (32%) in low IGF group *vs.* 22 (30.1%) in high IGF group, whereas patients with BMI (≥ 30) were 8 (32%) in low IGF group *vs.* 28 (38.4%) in high IGF group, with no significant difference between both groups ($p = 0.843$). Similar finding was reported by Tong et al[30] who reported no significant relationship between BMI and IGF among high and low IGF groups. On the other hand, Tong et al[30] found that high levels of IGF-1 were associated with better RFS in non-overweight patients but worse outcomes in overweight patients.

IGF-1 release from differentiated or precursor adipocytes produced by obese patients was approximately two times higher than in lean people. Additionally, it promoted the growth of MCF7 cells in co-culture studies[31,32], confirming the idea that obesity may contribute to the development of breast cancer. The results

of the current study may have been influenced by the smaller sample size. However, it was determined that there was a significant difference between the high IGF group and the low IGF group in terms of response. This may be related to the increased number of patients with progressive disease in high IGF group than low IGF group. Furthermore, it was found that, as previously noted, there was a correlation between progressive disease and increased IGF levels because IGF is regarded as a good indicator of progression and metastasis[28].

Additionally, when comparing the PFS of the two groups (low IGF, high IGF), the results showed that the low IGF group had a median of PFS at 1 year of about 60.0 ± 9.8% *vs*. 12.3 ± 3.8% in the high IGF group. This difference between the two groups was statistically significant ($p = 0.000$), indicating that MBC patients with low IGF have better PFS than MBC with high IGF. Up till now no studies are available to compare our results with. Tong et al[30] found there was no significant difference in 4-year recurrent free survival (RFS) between the low and high IGF-1 groups.

Regarding to the role of IGF-1 in proliferation of breast cancer, there was no significant difference in the IGF level between progressive and non-progressive group at baseline. While after 6 months, the mean of IGF in the progressive group was significantly higher than the non-progressive group. Additionally, the mean IGF concentration in progressive patients reported a significant increase after six months of treatment ($p^2 = 0.000$). This may imply that the higher the IGF level, the greater the activation of IGF-IR, resulting in the increased proliferation and metastasis of breast cancer[28].

Conclusions

Metformin as an adjuvant therapy has no effect in reducing the level of IGF-1 among nondiabetic MBC patients. IGF can be considered as a significant predictor of disease progression in patients with metastatic BC. Consequently, lower levels of IGF-1 are associated with higher levels of PFS and RR.

Ethics Approval

The Faculty of Pharmacy's Research and Ethics Committee (REC), Beni-Suef University approved to conduct this study with ethical number (REC-H-PhBSU-21028). The study was carried out following the good clinical practices stated by the Declaration of Helsinki.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Funding

This research work was funded by the Institutional Fund projects under grant No. (IFPIP: 1831-141-1443). Therefore, the authors gratefully acknowledge the technical and financial support from the Ministry of Education and King Abdulaziz University (KAU), Deanship of Scientific Research (DSR), Jeddah, Saudi Arabia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

Conceptualization, methodology, resources, visualization: Marian S. Boshra, A. Gabr; Supervision: Heba F Salem, Marian S. Boshra, Marwa O. Elgendy, software, validation, investigation, writing–original draft preparation: Nourhan M. Essa; writing–review and editing: Marian S. Boshra; Reviewing, editing, data curation, formal analysis, funding acquisition: Steve Harakeh, Maged Mostafa Mahmoud, Asmaa Alharbi, Hanaa M. Tashkandi. All authors have read and agreed to the published version of the manuscript.

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

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